1969 CONFERENCE ON CITRUS CHEMISTRY AND UTILIZATION

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ABSTRACTS OF PAPERS

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THE EFFECTS OF STORAGE CONDITIONS ON THE LIPID COMPOSITION OF COMMERCIALLY PREPARED ORANGE JUICE

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The role of lipids in off-flavor development in processed orange juice has been the subject of numerous investigations from this Laboratory. Previous storage studies conducted on orange lipid fractions were essentially qualitative and did not take into consideration the dynamic relationship of these fractions. The present study was undertaken to quantitatively follow these fractions during prolonged storage at adverse temperatures.

Studies conducted on orange juice chilled in glass containers were obtained from a local packer. The samples were divided equally and stored at 40°F. and 85°F. Total lipids were extracted from each juice and fractionated on a silica gel column into neutral and polar lipids. The neutral and polar lipids were, in turn, subfractionated into their individual components.

Over a storage period of 15 months, the percentage of neutral lipids increased from 29.5% to 35.2% in 40°F. juice while this fraction in 85°F. juice increased to 54.3%. Quantitative TLC of the neutral lipids revealed the following information shown in Table I. The free fatty acid percentage increased in the 40°F. juice from 14.7% to 26.2% while the 85°F. juice showed an increase to 54.9%. Gas chromatographic analyses of these acids at 3 and 15 month storage is shown in Table II. Table II shows a definite increase in the di- and triunsaturated acids upon extended storage at 85°F. The polyunsaturated fatty acids are most susceptible to oxidative deterioration. It is, therefore, not surprising that with the enhanced liberation of unsaturated acids at 85°F., there is also an increased propensity to form off-flavor compounds.

The lipids which show enhanced enzymic hydrolysis at elevated temperatures, and thus, liberate free fatty acids, have been traced to the polar lipid fraction. Phospholipids, which comprise a large percentage of the polar lipid fraction, have been reported by a number of food product investigators to be highly prone to enzymic hydrolysis (phospholipase activity). Estimation of the phospholipid concentration by phospholipid phosphorus analyses showed that after 15 months in storage the total phospholipid phosphorus content of 15 g. powder from 40°F. juice was 73.5 μM while this value decreased to 25.8 μM in 85°F. juice. With loss in phospholipid phosphorus there is a concomitant gain in free fatty acids. The phospholipids most susceptible to breakdown at elevated storage temperatures are phosphatidyl choline, phosphatidyl ethanolamine, and phosphatidyl inositol.

TABLE I. PERCENT COMPOSITION OF NEUTRAL LIPIDS AS FUNCTION OF STORAGE TIME AND TEMPERATURE

	Time (Mo.)						
	0	5		11		15	
		40°F.	85°F.	40°F.	85°F.	40°F.	85°F.
Component							
Monoglyceride	1.5	1.7	1.4	0.7	1.2	0.7	0.3
Fatty acid	14.7	21.7	45.6	24.8	49.6	26.2	54.9
Sterol	26.5	24.2	17.1	24.6	15.7	27.1	15.3
Diglyceride	6.3	6.0	2.5	3.9	1.2	3.4	2.0
Triglyceride	20.5	22.9	12.5	23.5	14.1	23.7	12.3
Steryl ester	20.5	18.6	13.3	14.6	10.3	16.2	10.9
Hydrocarbon	9.2	4.9	7.1	6.8	7.4	2.4	4.3

TABLE II. COMPOSITION OF FREE FATTY ACIDS AS FUNCTION OF TIME AND TEMPERATURE.

		Fatty Acid *(Mole %)						
Storage Time (Mo.)	Temp.	16:0	16:1	16:2 & 18:0I	18:0	18:1	18:2	18:3
3	40°F.	27.0	5.6	1.4	3.6	26.3	23.0	13.1
3	85°F.	27.4	4.3	1.4	2.3	24.6	26.0	14.0
15	40°F.	27.7	3.6	1.5	2.6	26.8	25.5	12.3
15	85°F.	24.8	4.4	1.8	3.1	24.0	27.7	15.2

* No. of Carbon Atoms: No. of Double Bonds

COMPOSITION AND INHERITANCE OF FLAVANONES IN CITRUS FRUIT

by

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Swingle, in his comprehensive work on citrus taxonomy, recognized the possible usefulness of a knowledge of flavonoid composition in serving as an aid in differentiating between different species of Citrus. He states that "the nature of the characteristic glucoside present in the tissues of a species of Citrus may be of definite taxonomic significance in distinguishing that species from another species to which it may have superficial resemblances."

Kefford grouped some of the citrus fruit (sweet oranges, mandarins, lemons, and citrons) together due to the presence of hesperidin as their principal flavonoid; and others (pummelo and grapefruit) in a group where naringin was the principal flavonoid. He considered the presence of both hesperidin and naringin in the Natsudaidai (a suspected mandarin-pummelo hybrid) to be significant as it represented a possible combination of the two groups.

Horowitz, in discussing the occurrence of flavanone rutinosides and neohesperidosides in citrus, was led to conclude that it was likely that most citrus species contained either all rutinose derivatives or all neohesperidose derivatives.

Despite the early recognition of the possible usefulness of flavanones as taxonomic markers in citrus, no systematic study on a wide selection of citrus species and varieties has been made prior to our own survey.

Accidental hybridization and preservation of progeny through nucellar embryony has led to the establishment of hybrid populations of citrus which were both geographically and morphologically remote from parental types. Morphological characteristics alone are often not sufficient to identify parent-progeny relationships and consequently many hybrids have been assigned species status.

Experimental citrus breeding studies are frustrated by nucellar embryony, incompatibility, and self-sterility, as well as the limitations of time, space, and availability of funds necessary to support a lengthy breeding program. However, for the more economically important species of citrus, such breeding programs have been undertaken, and have yielded valuable information concerning the taxonomic relationships of citrus.

Two modern "systems" of citrus taxonomy have been published. The most widely cited system is that of Swingle, which has recently been brought up to date by Reece. The other is that of Tanaka. Hodgson has

summarized and criticized these two systems and has suggested the use of an intermediate system for the genus Citrus consisting of 36 species instead of the 16 recognized by Swingle or the 157 proposed by Tanaka. Many forms which are considered by Swingle to be botanical varieties, hybrids, or even cultivars are given species status by Tanaka. The concurrent use of names from the two systems, or even older systems, by citrus breeders, horticulturists, food technologists, or chemists has led to confusion and misinterpretation, especially among those unfamiliar with citrus taxonomy.

In reviewing the chemical literature, it becomes apparent that many chemical investigations on citrus have very limited value to chemosystematics due to the ambiguous manner in which the plant material was identified. The somewhat naive faith some investigators have in the identification of fruit available on the commercial market may in part, explain some discrepancies found in the literature on citrus composition. False cognates in different languages can also lead to confusion. For instance, the French often refer to fruit of C. limon as a citron, while in English the same fruit is called a lemon and the name citron is reserved for the fruit of C. medica.

Rather than attempt to make subjective evaluations of all the prior research concerning the presence or absence of flavanones in various citrus species, it is more rewarding to apply newer and more rapid analytical methods to authenticated plant material as both a check on the prior work and to enable a greater number of varieties to be investigated. The simplified assay method employing polyamide thin-layer chromatography developed in cooperative research by the University of Oklahoma Research Institute and the Food Crops Utilization Research Laboratory at Weslaco provides a rapid means of identifying the probable presence of the citrus flavanones in extracts of the plant material. Such a rapid means of flavanone analysis is needed if the chemosystematics of citrus flavanones is to have practical usefulness for the citrus breeder and the taxonomic botanist. A knowledge of the presence of flavanones characteristic of a particular species may also be useful in determining the species composition of citrus products in the processing industry.

Our survey included 41 citrus varieties representing 18 recognized species of citrus, and also 49 hybrids of 18 different crosses.

A representative sample of one or more mature fruit was collected from each tree of an authenticated variety collection. The whole fruit was cut up and placed in boiling isopropanol which was then brought to a second boil. The fruit-isopropanol mixture was then reduced to a homogeneous appearing slurry in a blender. The slurry was filtered and the filtrate allowed to stand at 5°C. for at least 1 week. After this time the resulting clear amber solution was then analyzed by thin-layer chromatography.

The chromatograms were documented by photographing them in the dark while being illuminated by long-wave ultraviolet light. Photographs were made immediately after chromatogram development and drying; again after spraying with 1% $AlCl_3$ in methanol; and a third after spraying with Neu's Reagent.

Flavanone spots fluoresced a bright greenish-yellow with $AlCl_3$ spray. Flavones were present between naringin and the origin which also gave a yellow fluorescence with $AlCl_3$ and often prevented an unequivocal assessment of the presence or absence of the eriodictyol-7-rhamnosyl-glucosides or of the naringenin-4^1-glucoside-7-rhamnosylglucosides. With Neu's Reagent these flavones generally gave a visible yellow color and exhibited enhanced fluorescence intensity.

Thus, six of the flavanones: naringin, naringenin-7-rutinoside, poncirin, isosakuranetin-7-rutinoside, neohesperidin, and hesperidin were determined with a high degree of accuracy by chromatography on the polyamide resin with a nitromethane-methanol solvent system.

It became apparent upon examining the chromatographic flavanone pattern of the various citrus species and varieties that quantitative as well as qualitative differences existed between individual taxa. The relative proportion of each flavanone within any sample could be estimated by examination of the fluorescence-intensity of the individual component spots on the chromatogram. The most intensely fluorescent flavanone spot among those identified on the chromatogram of a fruit sample was assigned an arbitrary value of ten. All other flavanone spots in the same sample were rated in numerical values of 1 through 10 to express their fluorescence-intensity relative to the most intense spot.

Tables I and II illustrates some of the data obtained for a number of the cirtus taxa and citrus hybrids.

Of those species recognized by Swingle which were included in our survey, six contain exclusively flavanone rutinosides; while three: C. grandis, C. aurantium, and Poncirus trifoliata contain only the neohesperidosides.

The chromatographic pattern of known citrus hybrids as illustrated in Table II shows that when a species containing only flavanone rutinosides is crossed with another containing only neohesperidosides, then the progeny contain both types of flavanone glycosides. The alleles responsible for the production of these two rhamnosylglucoside isomers thus appear to involve additive inheritance in the F_1 generation.

All four taxa shown in Table I which contain both rutinosides and neohesperidosides of flavanones have been thought to be of probably hybrid origin.

Thus, the present survey further confirms the validity of Horowitz's observation that citrus fruit could be classed as containing either rutinosyl glycosides or neohesperidosyl glycosides. As a corollary to this, it appears then that those citrus types which contain both classes of flavanone glycosides, in general, can be considered as being a possible hybrid.

In the known hybrids which were analyzed, no examples were found where an aglycone was present which was not also known to occur in one of the parents. Thus, besides the additivity of alleles responsible for the rhamnoglucoside type, F_1 progeny of interspecific crosses show an additivity of alleles for the nature of the aglycones present.

In those taxa where more than one variety were analyzed it is apparent that not only a general qualitative consistency of flavanone composition exists but also the relative amounts of flavanones remains essentially constant.

When one examines the relative fluorescence-intensity values of flavanones among hybrids of C. paradisi and C. reticulata we find frequent reoccurrences of the values characteristic of C. reticulata and C. sinensis. If our prior deductions are correct that inheritance of the glycosidic patterns among F_1 generations is based on additivity of glycoside alleles, as well as additivity of aglycone alleles; then the absence of neohesperidosides and the presence of the typical C. sinensis and/or C. reticulata relative fluorescence-intensity values argue in favor of C. paradisi being heterozygous with respect to both glycosidic and aglycone alleles.

Since other C. paradisi x C. reticulata hybrids, such as 'Satsumelo', show a relative fluorescence-intensity pattern typical of C. paradisi aglycones yet are completely devoid of neohesperidosides, it is suggestive of independent transmittance of the glycosidic allele and the aglycone allele(s). Further evidence of genotypic dihybridity of C. paradisi with regard to aglycone and glycosidic components is present in the 'Sunshine' tangelo. This has the aglycone fluorescence-intensity pattern typical of C. sinensis and/or C. reticulata but it still possesses a high percentage of neohesperidosides which apparently were inherited independent of aglycone alleles from C. paradisi.

The results of the present survey appear consistent with the hypothesis that glycosylation occurs late in the biosynthetic sequence of flavonoids, and also suggests that the glycosylation enzyme responsible for the introduction of rhamnosyl unit is nonspecific for the flavanone aglycone.

By employing the general concepts of flavanone inheritance which we have deduced from this survey it may be possible to aid both the

taxonomist and plant breeder in deducing the parentage of suspected hybrids by examining their flavanone composition.

The efforts by the breeder to produce cold-hardy hybrids by crossing P. trifoliata with C. sinensis has largely produced citranges with a high degree of bitterness. Since the major cause of bitterness in fresh fruit is due to the presence of flavanone neohesperidosides, this is not surprising. If the cold-hardiness of P. trifoliata is inherited independent of the allele responsible for the production of neohesperidosides, then more palitable cold-hardy crosses are likely to result from backcrossing the citranges to C. sinensis. It may be possible to exercise some control of bitterness in hybrids by selecting potential crosses with due consideration to their neohesperidoside content.

TABLE I

RELATIVE FLUORESCENCE--INTENSITY OF FLAVANONES
WITHIN EACH FRUIT SAMPLE OF CITRUS TAXA

	7-Rutinosides					7-Neohesperidosides				
	Nar	Iso	Eri	Hes	Nar4	Nar	Iso	Eri	Hes	Nar4
Citrus sinensis	4	2		10	1	-	-		-	-
C. reticulata	4	2		10	-	-	-		-	-
C. aurantifolia	-	-		10	-	-	-		-	-
C. medica	10	-		8	5	-	-		-	-
C. limon	2	-	10	10	-	-	-	-	-	-
C. tachibana	1	T	-	10	-	-	-	-		-
C. grandis	-	-	-	-	-	10	-	-	-	1
C. aurantium	-	-		-		10	-		10	
C. paradisi	4	1		Γ	1	10	2		T	1
C. taiwanica	3	T		3		10	2		10	-
C. amblycarpa	3	1		10	-	3	1		10	-
Poncirus trifoliata	-	-	-	-	-	10	10	-	-	-
'Ponderosa lemon'	1	-	-	1	-	3	-	-	10	-

Symbols: Nar = Naringenin
Iso = Isosakuranetin
Eri = Eriodictyol
Hes = Hesperetin
Nar4 = Naringenin-4'-glucoside

TABLE II

RELATIVE FLUORESCENCE-INTENSITY OF FLAVANONES
WITHIN EACH FRUIT SAMPLE OF CITRUS HYBRIDS

	7-Rutinosides					7-Neohesperidosides				
	Nar	Iso	Eri	Hes	Nar4	Nar	Iso	Eri	Hes	Nar4
C. sinensis X P. trifoliata 'Troyer'	4	4		1		10	10		2	
C. limon X P.trifoliata	10	2		2		5	8		1	
C. grandis X 'Temple Tangor'	3	-		1	-	10	1		9	-
C. paradisi X C. reticulata										
'Minneola'	4	2		10	-	-	-	-	-	-
'Satsumelo'	10	2		T	-	-	-	-	-	-
'Sunshine'	1	T		2		4	3		10	-

9/11/69

APPLICATIONS OF ENZYME RESEARCH TO CITRUS PROCESSING

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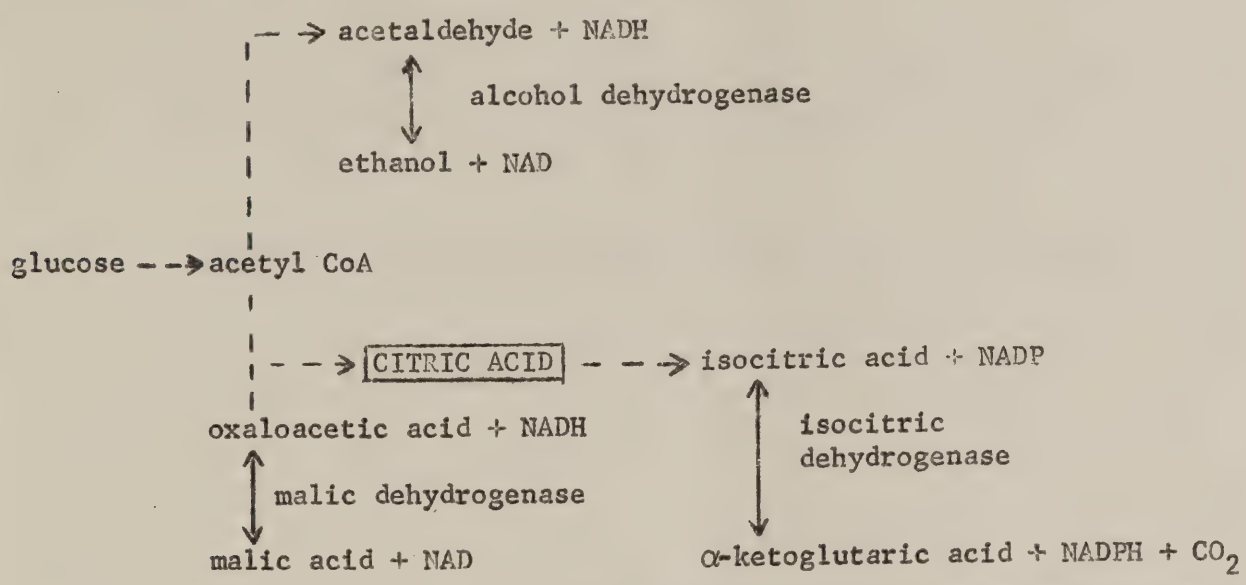
Our research on enzyme reactions in citrus has provided leads toward solving two important problems in citrus processing. By defining the substrate requirements for the enzymic synthesis and degradation of citric acid, we now understand how acidity can be controlled in citrus fruit. We are using this information to develop a method that would significantly reduce the total acidity of orange and grapefruit juice. By separating the role of pectin in "cloud" stabilization from its role as substrate for pectinesterase, we have been able to devise an approach toward stabilizing orange and grapefruit juices without heating.

DECREASING ACIDITY. We observed that in orange and grapefruit juice vesicles, the oxidation-reduction coenzyme, nicotinamide adenine dinucleotide (NAD), becomes more reduced as the fruit matures and the acidity declines. Since the oxidized form of the coenzyme is required for the enzymic oxidation of malic acid to oxaloacetic acid in the biosynthetic pathway to citric acid, it occurred to us that synthesis of citric acid was probably depressed by the reduced coenzyme and that the lower acidity of mature fruit reflects this slower rate of synthesis.

Since the normal reoxidation system for reduced NAD is respiratory-O_2, we examined anaerobic treatment as a method for depressing the concentration of the oxidized form of NAD and the synthesis of citric acid. Oranges and grapefruit were allowed to metabolize in the absence of O_2 at 92°F. and 104°F. for 20 hours. Table I shows the change in coenzyme redox ratios, Brix, acidity, and ethanol content of juice from these fruit. NAD was in a more reduced state in juice from N_2-treated fruit. Juice from N_2-treated fruit also had a higher Brix to acid ratio and showed more resistance to natural clarification. The peel could be removed easier from anaerobic-treated fruit than from the air-treated, and less albedo adhered to the endocarp of the N_2-treated.

The increase in ethanol in the treated fruit is evidence that acetyl CoA is diverted from citrate synthesis to acetaldehyde and ethanol. This diversion supports the interpretation that the synthesis of citric acid is blocked. Degradation of citric acid probably proceeds at the same rate or more rapidly, since the NADP-redox ratio decreased, which would increase the rate of the isocitric dehydrogenase reaction. The relationships between citric acid, coenzymes and ethanol are shown in figure 1.

Figure 1. Biosynthesis and degradation of citric acid in citrus fruit vesicles.



These results suggest that the acidity of citrus fruit can be lowered after harvest and before processing by subjecting intact fruit to a short anaerobic treatment. The degree of anerobiosis, temperature and duration of treatment remains to be established for maximum acid reduction.

CLOUD STABILIZATION. Citrus juice-soluble-pectin is considered to be responsible for natural clarification of citrus juice. Demethylated by pectin esterase (PE), the pectin forms insoluble complexes with Ca^{++} and "breaks" the natural fruit juice colloid.

Although synthetic and natural gums are used to establish colloidal suspensions in citrus beverages, the need for pectin in natural juice was questioned because of our observation that a "cloud" could be developed in water with a high-speed centrifugate from orange juice.

Table II shows that the "cloud" was stable when developed in water or in synthetic serum without pectin but not when developed in natural serum and in synthetic serum with 0.03% pectin. These results suggested to us that removing pectin would be an alternative to heating to depress PE activity.

As a polymer, pectin can be removed from solution by solvent or salt precipitation. However, pectin also can be depolymerized by polygalacturonase (PG) so that it no longer can form a precipitate with Ca^{++}. Table III shows that orange juice serum treated with a commercial enzyme, high in

PG activity, is a stable medium for orange juice "cloud." Thus, depolymerization of pectin with enzymes is effective in stabilizing orange juice and probably other citrus juices.

These observations suggest that alternatives to heating can be found for cloud stabilization. Heating also stabilizes flavor. Examination of the enzyme reactions involved in flavor degradation may provide alternatives to heating for suppressing these reactions as well.

TABLE I. RESPONSE OF ORANGE AND GRAPEFRUIT TO ANAEROBIC METABOLISM.

	Oranges, 90°F.		Grapefruit, 104°F.	
	Air	N_2	Air	N_2
$\frac{NADPH}{NAD}$	0.26	0.38	0.53	1.02
$\frac{NADPH}{NADP}$	2.05	1.41	2.31	1.54
ACIDITY CITRIC ACID %	1.28	1.13	1.48	1.38
°BRIX	10.55	10.38	9.19	9.69
°B/A	8.24	9.19	6.2	7.0
ETHANOL µMOLES/ml	3.8	20.0	0.8	13.1

Gas was passed at rate of 1 l/hr. for 20 hrs. through 7-gallon pails containing oranges or grapefruit at 90°F. or 104°F.

TABLE II. CONTRIBUTION OF MEDIUM TO STABILITY OF ORANGE JUICE CLOUD.

Cloud resuspending media	Cloud units °/oo B	
Serum	0.29	Clarified
H_2O	1.30	Not clarified
Synthetic serum	1.12	Not clarified
Syn. serum plus 0.03% pectin	0.28	Clarified

Orange juice cloud was separated from serum by centrifuging at 100,000 x g. for 30 minutes. The cloud was resuspended in media at the original concentration. Synthetic serum contained 0.4% KCl, 100 ppm Ca^{++}, 13% sugar (2 sucrose: 1 glucose: 1 fructose), 1% anhy. citric acid, pH 3.7. Cloud stability is expressed in Cloud Units (ARS-72-8. pp thousand of 325 mesh bentonite equivalence in light transmission). Light transmission after 15 days at 4°C. was measured spectrophometrically on centrifuged juice (340 x g. for 10 minutes) and converted into cloud units. Sodium benzoate at 0.1% was added to all samples to retard microbial clarification.

TABLE III. STABILIZATION OF UNHEATED ORANGE JUICE WITH ENZYMES

	Cloud Units (°/oo B)	
Untreated	0.26	Clarified
Treated	1.3	Not clarified

See Table II for details of separating serum from orange juice and measuring stability of reconstituted juice. Treated juice was reconstituted from cloud and from serum that was treated 18 hours at 4°C. with 500 ppm. Klerzyme 200 (Wallerstein Co.).

10/14/69

RECENT DEVELOPMENTS IN SRS CONSUMER RESEARCH ON CITRUS PRODUCTS

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As many of you know, the Special Surveys Branch of the Statistical Reporting Service (SRS) is composed of a small group of social scientists. Like the other groups in SRS, we are data collectors, but we specialize in data on attitudes and opinions about farm products. And we generally talk with the consumer rather than the farmer. We are interested in learning about reactions at the household level which affect the marketing and consumption of farm products--or the potential for new products developed by the department's utilization researchers.

My topic today is the research we've been doing in the past year or so on orange juice. Much of this work has been sensory evaluation, more popularly known as "taste-testing," using products and financial assistance from the Florida Citrus Commission, the Citrus Experiment Station, and the ARS Fruit and Vegetable Products Laboratory at Winter Haven. We believe that taste-testing is a valuable first step in assessing product variations or new products. It can help to determine whether there is any hope for a processing innovation, and which variations in a product, such as degree of sweetness, are most appealing to consumers. And it is a relatively inexpensive procedure compared to some other research approaches. However, taste tests must be interpreted with caution, and these data cannot substitute for data from such other methods as concept testing, household placement tests, market tests, and opinion studies in reaching an overall evaluation of a product's chances for success in the market place.

Our sensory evaluation laboratory in Washington, D. C. was built about 6 years ago. It was patterned after the facilities and procedures then used by the Quartermaster Food and Container Institute for the Armed Forces, but since that time we have gradually adapted our methods to USDA needs as we became aware of improvements we could make.

The laboratory provides an environment in which people's reactions to products can be ascertained under controlled conditions. For example, subjects are isolated in booths to prevent social factors, such as the opinions of others, from influencing them; the product samples are all served at the same temperature; the order in which samples are presented is varied to equalize the effect of serving position on ratings; lighting is controlled to minimize differences in appearance among samples, to allow for recovery of physiological sensitivity between tasting one sample and the next, timed delays are introduced between sample presentations, rinse water is provided, and unsalted wafers or plain white bread may be given between samples.

The subjects for our experiments are selected from a list of about 500 department employees who have agreed to participate in the research program. We use as few as 24 people or as many as 100, depending on the research problem. These people have not been screened for taste sensitivity, they have not been trained as "tasters," they do not have expert knowledge of the products they judge, and they are not aware of what the differences are between the samples they are evaluating. In other words, our "tasters" have been chosen to simulate the reaction of ordinary consumers.

For preference tests, from 2 to 6 orange juice samples are served one at a time to each subject. About 2 ounces of each juice are served. The subjects indicate their opinion of each sample by giving a rating on the standard 9-point hedonic scale and writing in comments if they wish. Because the average or mean scores obtained in this manner can not be treated as absolute values, we are primarily interested in the relative scores for the different products.

For discrimination tests, we use the "triangle" method, in which the subject is served 3 samples simultaneously--two alike and one different. The subject selects the one sample he or she thinks is different from the other two.

Now for a brief resume of some of our taste-test data. The most striking finding in many of our experiments centers upon the Brix-acid ratio level of the orange juices tested, whether they were canned, frozen concentrates, or "instant." Within the usual range of sweetness levels, almost invariably the higher the Brix-acid ratio the higher the preference rating. Incidentally, this also seems to be true of other juices and drinks we have tested. It does not appear to matter greatly whether the orange juice is naturally sweet or is sweetened with either sucrose or calcium cyclamate. Our findings suggest that, to appeal to the "average" consumer in a laboratory taste-test situation, juices with a Brix-acid ratio of less than 17.0 should be sweetened. The sweetening agent may be either sucrose or calcium cyclamate, whichever is more desirable on the basis of other considerations. However, the sweetening process should not be carried as high as a Brix-acid ratio level of 24.0. Rather, the optimal appears to be between 17.0 and an undetermined level lower than 24.0. It was about 19.0 among one group of frozen concentrated orange juices we tested, but relatively little work has been done in our lab with juices between 19 and 24 Brix-acid ratios which would enable us to speculate about the desirability of juices with a ratio of 20 or 22.

Experiments were also run recently with four frozen concentrated orange juices to investigate reactions to variations in degrees of Brix. The Brix levels tested were 11.8, 12.8, 14.0, and 15.4, all at a Brix-acid ratio level of 17. The preference ratings indicate that the two middle Brix levels (12.8 and 14.0) were liked best, receiving almost identical mean scores. The top Brix level (15.4) scored somewhat lower--though not significantly lower--but the drop-off was not as great as some citrus experts might expect.

The highest Brix level also held a slight--though again not significant --edge over the lowest (11.8), which was the least appealing of these four juices to our subjects.

Discrimination tests were also conducted with the same four juices. Each juice was tested against all the others for a total of six pairings, and each of the pairings was tried by a different group of twenty-four subjects. Each subject participated in two triangle tests at the same sitting, tasting the same combination of juices, though not in the same serving pattern. Analyses were made separately for the first and second triangle tests, as well as for the results of the replicated triangle session for individual subjects. In a triangle test one-third of the subjects could be expected to make the correct selection by chance alone, while in a replicated triangle session, one-ninth of the subjects could make the right choice both times by chance alone. We found that regardless of the particular combinations of juices presented, usually only about half of the subjects made a correct selection of the "odd" juice, and only about one-fourth were correct in selecting the "odd" juice in both halves of the session. It is obvious that in a laboratory session many people cannot readily detect differences in Brix level, even between the extremes used in this experiment. The results were barely significant for the three pairings which involved juices more than one step apart on the Brix level continuum studied, but they were not significant for the three pairings of juices with adjacent Brix levels. Since the lack of statistical significance may have been due to the relatively small numbers of subjects who tried each pairing, we are repeating some of the pairings with additional subjects to maximize the opportunity for significant differences to show up.

A recent series of tests was conducted on essences in instant orange juice. Juices with several variations in essences developed by the Winter Haven U. S. Fruit and Vegetable Products Laboratory and a commercially available essence were tested against a control containing no essence. We found that there were no significant differences in mean preference scores among any of the essences, but that all of the juices containing essence were preferred significantly to the control product.

The most ambitious taste-testing project in which we have been involved is an investigation of relative preferences for frozen concentrated orange juices containing varying levels of peel oil. We wanted to know whether higher peel oil levels than those currently used would have any effect on consumer preference ratings. The study is being done with the assistance of a contractor who has had extensive experience in conducting taste tests with the same general procedures followed in our own lab. Tests were run concurrently during the summer of 1968 in our lab and in mobile laboratories set up in two shopping centers in Chicago, Illinois. In Chicago, both adults and children 8-12 years of age participated in the experiment. We are particularly interested in this research because it provides an opportunity to appraise the extent to which our employee panel can be considered representative of a more nearly average group of consumers as well as residents of another geographic area. It also permits us to compare the preferences of young children--who we cannot conveniently include in our own testing--with those of adults.

In both Chicago and Washington, orange juices at low, medium, and high natural sweetness levels were used. Within each sweetness level, 4 peel oil levels at equal intervals were tested. The lowest (approximately .015 volume percent) is somewhat below the usual commercial pack; the next (.030) is fairly typical of current industry practice; and the remaining two (.045 and .060) exceed this level.

In analysing the mean preference scores obtained from these tests, we found that the adults and children were in agreement, although the children tended to rate all the juices higher than the adults did. And there was also general agreement between adults in Chicago and those on our employee panel. However, the differences in mean scores from one peel oil level to the next tended to be more pronounced with the USDA group, perhaps because they have become familiar, through continued participation in our experiments, with taste-testing procedures and the scale that we use.

Overall, there was a generally linear downward trend in the mean preference scores for frozen concentrated orange juice with successively higher levels of peel oil. In other words, on the average, the more peel oil a juice contained, the less it was liked. This relationship was quite clear in the data from the USDA lab, where, as noted earlier, the differences in preference scores between peel oil levels were larger. However, in Chicago, the differences in ratings for the three lowest levels of peel oil included in the experiment were small, which might suggest that, within lower ranges, precise control of the amount of peel oil a product contains is not critical in maximizing consumer acceptance.

As a follow-up to this taste-testing, another research approach was tried this summer. We wanted to ascertain consumer evaluations of peel oil variations in orange juice as it is actually used in the home and to compare ratings under these conditions with ratings secured in lab tests. Three of the juices used previously were selected for this phase of the study--the medium sweetness juice with peel oil at .030, .045, and .060 volume percent. During a two-week test, these juices were presented one at a time to a group of households in Chicago within a ten-mile radius of the shopping centers in which the mobile labs had been placed. The homemakers were given a seven day supply of frozen concentrated orange juice and asked to serve it as they normally would. Each family member 8 years of age or over was asked to rate the product, after drinking it for about a week, on the same 9-point hedonic scale used in the laboratory tests. Some families were given .030 and .060, .030 and .045, or .045 and .060, in a balanced serving order, so that direct comparisons of the relative appeal of the variations could be made. These families were not aware of what the variations were. Other families received the same juice--either the .030, or the .045 or the .060--during both weeks of the test, so we'd know about reactions over a longer period of time. Tthey were not aware that they were trying the same juice both weeks. We also ran additional taste tests in the USDA lab with the same 3 juices.

So far we have only preliminary results from this project--unfortunately the experimental design that was necessary to provide answers to our questions has caused difficulties in applying appropriate

statistical techniques to analyze data. However, we believe it is safe to say that the household findings confirm the conclusions reached from the taste-tests: higher peel oil levels are not liked as well as lower ones. On the average, the reactions of those who used a given product for a two week period did not vary appreciably from the first week to the second, and appear to be in agreement with the reactions of those who were given two different products.

It is important to note that these evaluations were made on orange juice that had no off-flavors and had not been in storage very long. In commercial practice, it may be advisable to maintain a higher peel oil level than was optimum in these studies.

Another area of our research is survey among nationwide samples of consumers. A little over a year ago we did such a study on citrus, again with the assistance of a contractor. Interviewers visited a cross-section sample of about 2000 homemakers throughout the country. The interview lasted about an hour, and included many questions on homemakers' opinions and usage patterns for oranges, grapefruit, and lemons, and products made from these fruits.

Today I shall only discuss some of the data on orange juice. Please bear in mind that my comments are preliminary, of necessity, and are therefore subject to revision when we have completed our analysis and double checked everything for inclusion in the full report to be published next year.

One series of questions was devised to find out what homemakers think of orange juice. How would they really like it to be? They were asked to select from various alternatives those that best described what the ideal orange juice for their family should be like. All in all, we found considerable disagreement among respondents about each of the alternatives presented.

In response to a question about whether they would prefer orange juice with or without pulp, a slight majority said that the ideal orange juice for their family should be smooth--that is, it should contain no orange pieces or pulp--but a substantial proportion of the homemakers indicated that a juice which contained some orange pieces or pulp would be better for their households.

Homemakers were also asked if they would like orange juice sweetened or unsweetened. A little over half said they preferred an unsweetened orange juice. Those who said the ideal juice for their family should be sweetened were somewhat more likely to want it sweetened with sugar rather than with a low-calorie sweetener. Homemakers were fairly evenly divided in their preferences for a concentrate or a ready-to-serve orange juice and also in their preferences for a frozen or non-frozen form.

It's evident that no one orange juice product could meet all the various combinations of attributes that are desired by consumers. In fact none of the possible combinations of these attributes were preferred by as many as one homemaker in five.

Another series of questions was included on three new natural orange products of interest to the U. S. Fruit and Vegetable Products Laboratory, Winter Haven, Florida. Interviewers said:

"Here are a few questions about new products made from real oranges--some are available in some parts of the country and others are being considered."

"Please look at the items on this list, then tell me which of these products you would be interested in."

The list read:

Instant orange juice--powder or crystals that will dissolve in water to make real orange juice.

Real orange juice in solid form that can be chewed or melted in the mouth.

Orange flavored topping in pressurized cans for use on cakes, pastries or ice creams.

No attempt was made to inform homemakers about how these products might be packaged or priced.

Homemakers reacted most favorably to the idea of instant orange juice and least favorably to orange juice tablets. Roughly 6 in 10 expressed an interest in instant orange juice, 4 in 10 were interested in orange flavored topping, and only 2 in 10 thought real orange juice tablets were appealing. Those who were interested in these product concepts were largely motivated by a feeling that they would be easy to prepare and serve.

These questions on homemakers' opinions, product placement in the homes and taste tests in the laboratory all point up utilization research areas that should be further explored to increase consumers' satisfaction with and consumption of orange products.

BITTERNESS, SWEETNESS AND THE STRUCTURE OF CITRUS GLYCOSIDES

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Certain citrus fruits contain intensely bitter flavanone glycosides which have as their sugar component the disaccharide neohesperidose. Other citrus fruits contain tasteless flavanone glycosides in which the sugar component is the isomeric disaccharide rutinose. Neohesperidose and rutinose are composed of one molecule each of L-rhamnose and D-glucose. In neohesperidose the rhamnose is attached to the C-2 hydroxyl group of glucose, while in rutinose the rhamnose is attached to the C-6 hydroxyl group of glucose. (In both cases the glucose is linked to the flavanone aglycone when the disaccharides are glycosidically bound.) Since the bitter and tasteless flavanone glycosides are identical in every respect except the point of attachment of rhamnose to glucose, it follows that this structural feature is of prime importance in determining bitterness or tastelessness in this group of compounds.

To arrive at a better understanding of this phenomenon, various modifications have been made at selected sites in the bitter flavanone glycosides to see how the taste properties are affected. For example, conversion of a bitter flavanone neohesperidoside to the corresponding flavone neohesperidoside results in a tasteless compound. Of particular interest are the products obtained by hydrogenating the chalcone form of various flavanone glycosides to the corresponding dihydrochalcone. Several of these compounds are intensely sweet, including naringin dihydrochalcone, neohesperidin dihydrochalcone and hesperetin dihydrochalcone glucoside (HDG). Equations showing the structures and preparation of these sweeteners are given below (Neo = neohesperidosyl; Rut = rutinosyl).

The sweetness of the dihydrochalcones is many times greater than that of sugar but, compared with sugar, the dihydrochalcone sweetness is relatively slow in onset, is very persistent, and is characterized by some as having a licorice-like quality. The approximate equivalent sweetness of some of the dihydrochalcones compared with sodium saccharin is shown in the following Table:

	Equivalent Molar Concentrations	Equivalent Weights
Neohesperidin dihydrochalcone	1×10^{-5}	1
HDG	2×10^{-4}	15
Naringin dihydrochalcone	2×10^{-4}	19
Sodium saccharin	2×10^{-4}	7

In studies carried out by Dr. A. N. Booth at the Western Regional Research Laboratory there appeared to be no evidence of toxic effects when dihydrochalcones were fed to rats at 5% of the diet for as long as 170 days.

Reviews of this field and pertinent literature references can be found in the publications listed below.

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R. M. Horowitz, B. Gentili, A. N. Booth, and D. J. Robbins, ARS Publication CA 74-18, Western Utilization Research and Development Division, U. S. Department of Agriculture, Albany and Pasadena, California, 1968

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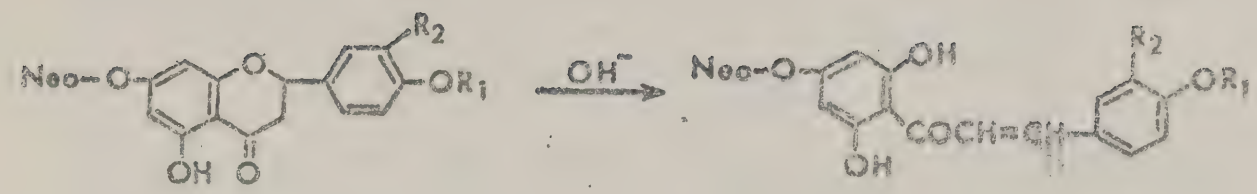
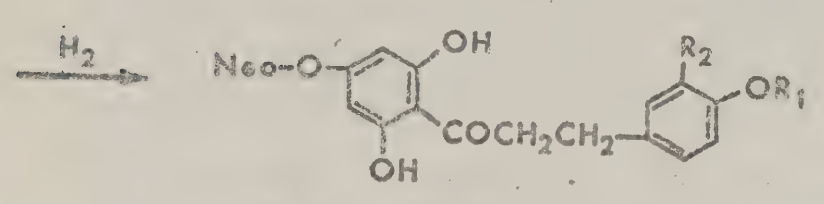
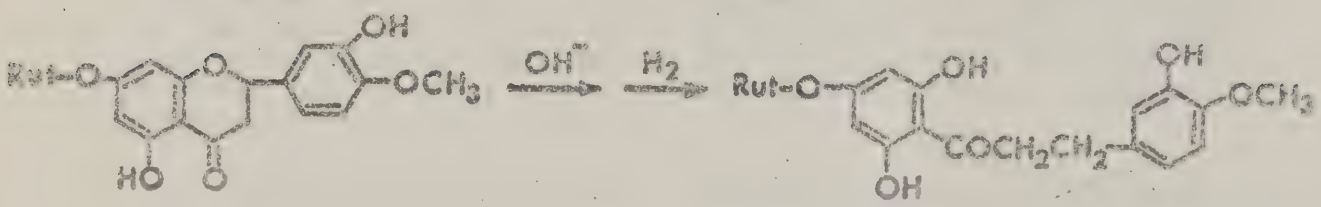


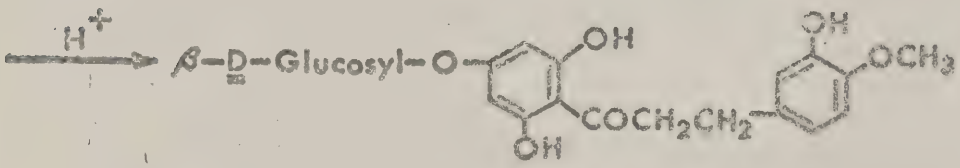
1. Naringin: $R_1 = R_2 = H$
2. Neohesperidin: $R_1 = CH_3$, $R_2 = OH$



1. Naringin dihydrochalcone
2. Neohesperidin dihydrochalcone



Hesperidin



HDG

9/4/69

ANALYSIS OF STORED INSTANT ORANGE JUICE AND TASTE EVALUATION OF SOME STORAGE DECOMPOSITION PRODUCTS

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Instant orange juice (IOJ) undergoes a flavor change after prolonged storage at elevated temperatures. This off-flavor development is attributed to nonenzymic browning products formed during storage.

Table 1 lists the 18 storage products we have identified from instant orange juice and their threshold levels determined in reconstituted instant orange juice. The type of contribution that each compound makes individually to off-flavor in orange juice is also noted in Table 1. However, in combination as they are found in juice, the flavor contribution might not be the same as when tested individually.

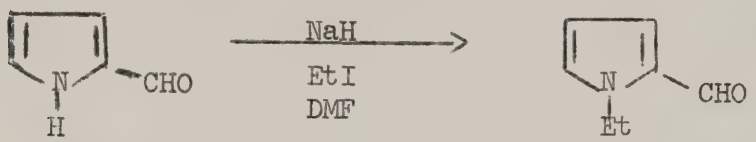
Taste threshold levels can also be affected by other compounds present. In one experiment, compounds 1, 2, and 3 of Table 1 were tested at half their threshold levels (1, 2.5 and 5p.p.m., respectively). This combination was detected by the taste panel at those levels. Most of the compounds caused a burned flavor when added to juice, although no one sample was quite like that of off-flavor powder. It is likely that the off-flavor is caused by a combination of these compounds in stored IOJ.

Two of the compounds in Table 1, 3-hydroxy-2-pyrone (5) and tiglic acid (7), are reported here for the first time as IOJ storage products. They were identified as the others had been - by gas chromatographic (GLC) separation of an extract from stored IOJ and comparison of the infrared and mass spectra, and thin-layer and GLC retention times of the separated compounds with authentic samples.

TABLE I. TASTE THRESHOLD VALUES FOR STORAGE PRODUCTS IN INSTANT ORANGE JUICE.

Compounds		Threshold (ppm)	Flavor contribution
1. N-Ethylpyrrole-2-carboxaldehyde		2	burned
2. Methylcyclopentenolone		5	burned
3. 5-Methyl-2-furfural		10	burned
4. Acetylformoin (4-hydroxy-2,3,5-hexane-trione)		18	bitter
5. 3-Hydroxy-2-pyrone		30	burned
6. Furfuryl alcohol		31	sour
7. Tiglic acid		60	burned
8. Furfural		81	burned
9. Benzoic acid		85	burned
10. Acetic acid		110	sour
11. Levulinic acid		110	burned
12. 5-Methylpyrrole-2-carboxaldehyde		110	burned
13. 2-Acetylfuran		110	burned
14. 2-Acetylpyrrole		200	burned
15. 2-Hydroxyacetylfuran	>	200	- -
16. 5-Hydroxymethylfurfural	>	200	- -
17. γ-Butyrolactone	>	200	- -
18. $C_6H_8O_4$	>	200	bitter

Eleven of these products were available commercially and were purified, when necessary, by distillation or preparative GLC. The other seven compounds were synthesized according to the following schemes:



(1)

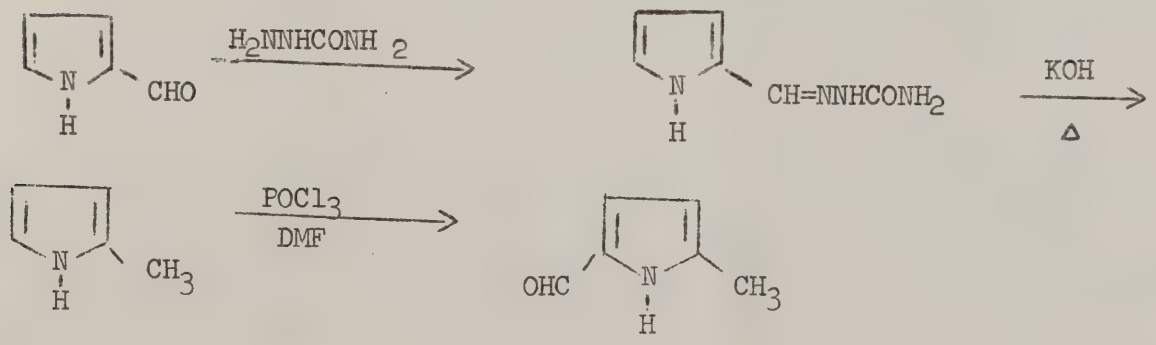
Acetylformoin (4)

Goto, Bull. Chem. Soc. Japan 36, 147 (1963)

Mucic acid

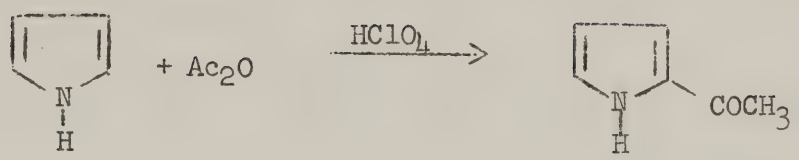
(5)

Wiley and Jarobe, J. Am. Chem. Soc. 78, 2398 (1956)



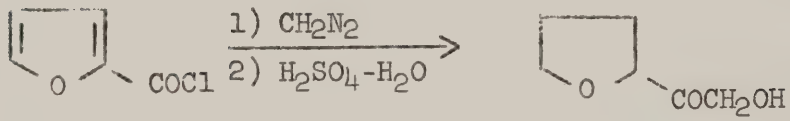
(12)

cf. Silverstein et al., J. Org. Chem. 20, 668 (1955).



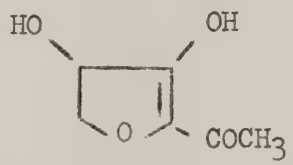
(14)

Berlin, J. Gen. Chem. (USSR) 14, 438 (1944).



(15)

Miller and Cantor, J. Am. Chem. Soc. 74, 5236 (1952)

D-glucose + CH_3NH_2 $\xrightarrow{HOAc}$ 

$C_6H_8O_4$

(18)

Compound 18 of Table 1 is identical to a compound that Severin and Seilmier [Z. Lebensm.-Unters. Forsch. 137, 4 (1968)] recently isolated from D-glucose-methylamine degradation. The structure which they assigned to this compound (shown above) is now in question. Further work on the structure of this compound is in progress at our laboratories.

We have made some attempts to quantitatively analyze off-flavor instant orange juice for some of these storage products. 5-Hydroxy-methylfurfural was not present in sufficient concentration to apply to reconstituted instant orange juice, the analytical procedure that had been used to detect this compound in honey [Winkler, Chem. Abs. 49, 16259b]. Extraction of instant orange juice to concentrate the 5-hydroxymethylfurfural and

other browning products has so far not given reproducible quantitative results. Efforts to analyze off-flavor instant orange juice quantitatively are continuing.

Storage stability of instant orange juice at 70°F. has been doubled by adding the carbonyl binding agent, sulfur dioxide. Carboxymethylcellulose (CMC) types 7AP, 7HP, and 7L2P have also been effective in increasing storage stability, although the mechanism by which they exhibit off-flavor development has not been determined.

9/10/69

LIMONIN IN FLORIDA CITRUS: A PRELIMINARY REPORT

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At the conference last year Dr. Vincent P. Maier, Laboratory of Fruit and Vegetable Chemistry, Pasadena, discussed the chemistry of Limonin. He and his co-workers had established the fact that limonin does not exist as such in normal, intact citrus fruit, but is formed by either enzyme activity or acid catalysis from non-bitter limonoate A-ring lactone upon disruption of the fruit.

Methods of analysis for limonin have only recently been developed. Chandler and Kefford (1), in Australia, developed a tedious technique involving formation of dinitrophenylhydrazone that took most of 2 days to complete. The Stanford Research Institute (2), under contract with our Pasadena laboratory, developed a procedure involving hydroxamation and acid ferric perchlorate color development, but it was subject to interference from substances other than limonin in fruit tissues and in all juices other than orange juice that were examined.

We used this SRI method to study the presence of limonin or its precursor in Valencia orange juices obtained from the maturity-extractor yield studies of the Florida Citrus Commission at Lake Alfred in the Spring of 1968. Although the SRI method gave fairly consistent recoveries of limonin added to orange juice, it was not reliable at concentrations below 10 p.p.m. About the only conclusion we could draw was that increased bitterness of juices developed by increasing extractor pressures from 16# to 45# was not due to increased extraction of limonoate lactone.

When Dr. Maier was here last October he described the fundamentals of a TLC procedure being developed at Pasadena for measurement of limonin. We used our adaptation of his method for the studies reported here. Since he has since improved many details of the procedure, and it is now in process of publication (3) we will not take time to present details of our procedure. In principle, we extracted the sample with methylene chloride, transferred the extracted material to acetonitrile, and spotted microliter aliquots on silica gel plates. We believe the results obtained are in the correct order of magnitude, and hope that work this season, using the improved method, will substantiate these findings.

Results of our analyses are shown in the following tables. Limonin (or more precisely, limonoate A-ring lactone) contents are shown in parts per million. Please bear in mind that 3 p.p.m. has been suggested as the approximate taste threshold of limonin in citrus juices.

TABLE I. FRESHLY EXTRACTED ORANGE JUICE FROM MATURITY-EXTRACTOR YIELD TESTS OF THE FLORIDA CITRUS COMMISSION, LAKE ALFRED.

Variety	Harvest date	Extraction pressure 16#	45#
Pineapple	2-19-69	0	0
	3-5-69	0	0
Valencia (C)	2-19-69	0.5	0.6
	3-5-69	0.3	0.3
	4-2-69	0	
Valencia (H)	2-19-69	2.5	1.2
	3-5-69	0	0
	4-2-69	0	0

While the juices extracted at 45# pressure were nearly always more bitter than those extracted at 16# pressure; there was no difference in limonin content. It is notable that the only positive tests for limonin occured before the fruit reached acceptable maturity for use in the manufacture of FCOJ.

TABLE II. JUICES FROM FRUIT HARVESTED MONTHLY AT THE ARS EXPERIMENTAL FARM.

Variety	Dec.	Jan.	Feb.	Mar.	Apr.	May	June
Hamlin	tr	0					
Parson Brown	1	0					
Temple	4	3	1	tr		0	
Pineapple	2				tr		
Murcott		8	5	3	5	1	tr
Valencia		1	2	0	tr	0	
Duncan		2	1	1	tr		
Marsh	7	3	3	3	1	tr	0

These juices were extracted by hand and immediately strained free of pulp, so that there was little opportunity to extract the precursor from tissue and convert it to limonin. The juice was held at -5°F. until tested. Note that the limonin content of all varieties decreased with maturity. Among the oranges, only the hybrids showed appreciable amounts of the bitter principle. The Murcott is notorious for its delayed bitterness. It is particularly interesting to note that Marsh grapefruit contained so much more limonin than the Duncan. Incidentally, in these particular samples, the naringin content of the Marsh was also higher. Undoubtedly, these two bitter components complement each other.

TABLE III. GRAPEFRUIT PEEL FROM ARS EXPERIMENTAL FARM.

	Dec. 66	July 67
Duncan	31	6
Marsh	156	12

The peel from hand reamed juice was held at -5°F. until used. Peel was comminuted with methanol and filtered. Solvent was substantially removed under vacuum and the aqueous residue extracted and tested for limonin as usual. Note that some of the bitter principle remained in the peel at late maturity.

TABLE IV. HYBRID TANGELOS AND TANGERINES PROCESSED INTO FCOJ IN THE PILOT PLANT.

Variety	Date Harvested	Limonin
K-Early	10/67	9
6-8-16	11/65	6
Lee	11/64	5
Lee	11/65	4
Osceola	11/64	4
Minneola	1/68	2
Page	11/64	2
Robinson	11/64	1
Orlando	11/67	tr
Nova	11/67	tr
Dancy	1/68	tr

In this series, the K-Early contained limonin in about equal proportions to the Murcott reported in Table 2. Selection 6-8-16, a deeply colored product of the breeding program of Crops Research Division, Orlando, will not be released for propagation because of its poor fruiting habit. The high values found for the Lee tangerine was surprising, as the flavor of this variety is usually rated quite high.

TABLE V. MISCELLANEOUS COMMERCIAL JUICES GRADED SUBSTANDARD BECAUSE OF BITTERNESS.

Chilled orange juice	Canned grapefruit juice
3	12
5	12
3	16
4	

Although the limonin contents of the orange juices were above the taste threshold, they were not sufficiently high to account for the highly objectionable bitterness. The grapefruit juice samples were canned in November-December, 1968, and contained unusually large amounts of limonin. These samples also contained above normal quantities of naringin, so undoubtedly both bitter principles contributed to their bitterness.

In summary, we have demonstrated differences in the limonin content of various Florida citrus fruits. It is important for both the production and processing industries to know these differences, but it is obvious that this particular component poses no great problem in Florida. In Australia and in California, published reports indicate that limonin concentrations up to 30 p.p.m. are no great rarity. It should also be pointed out that bitter flavor is influenced by accompanying acidity and sweetness, and that acceptable levels of limonin contents vary widely. It is to be hoped that a research contract recently granted by the Pasadena laboratory will delineate the problem more closely for all the citrus producing areas of this country. We will attempt to learn this season if rootstocks or other production variables will greatly affect limonin content of Florida citrus.

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2. Wilson, K. W., and C. A. Crutchfield. 1968. Spectrophotometric Determination of Limonin in Orange Juice. Agr. and Food Chemistry 16, 188-124.

3. Maier, V. P., and E. R. Grant. In Press. Specific TLC Assay of Limonin, a Citrus Bitter Principle.

LIQUID CO_2 EXTRACTION OF ORANGE ESSENCE

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Engineering and Development Laboratory
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The aroma constituents of orange juice and commercial orange essence are easily and completely extracted by direct contact with liquid carbon dioxide. Liquid carbon dioxide is a good solvent for most of the higher molecular weight organic compounds which are considered to be important in orange flavor, while the water is quite insoluble in the liquid carbon dioxide. Typical extraction takes place at room temperature and the corresponding equilibrium pressure of carbon dioxide, 800-900 psig. The carbon dioxide is separated from the aqueous phase and evaporated, leaving the extracted aroma constituents as product.

A previous presentation (Schultz, W. G. 1966. Liquid CO_2 for selective aroma extraction. Presented 26th Annual Meeting, Institute of Food Technologists, Portland, Oregon, May.) described the properties of CO_2 which make it desirable for essence stripping. Briefly, these include selectivity for aroma constituents, nontoxicity, and safety.

The extraction system used for the present work is the same unit that was described previously. It is designed for a 800-1000 psi working pressure and operates with either concurrent or countercurrent flow of liquid CO_2 and aqueous phase. The aqueous phase is circulated by diaphragm pumps, while the CO_2 is circulated without pumps. The CO_2 circulating section is equipped with stripping and condensing columns to vaporize the carbon dioxide, separating it from the product extract, and to recondense it to add back to the extraction system. Little of the extract is vaporized with the CO_2, for while extraction may proceed at 25° C, the CO_2 is vaporized at 30° C. Carbon dioxide may also be separated from the aroma constituents extract simply by reducing the pressure, since liquid CO_2 cannot exist at atmospheric pressure.

Just as in some extraction systems at atmospheric pressures, there were semilogarithmic relationships between distribution coefficients of a homologous series of organic compounds (between CO_2 and H_2O) and the number of carbon atoms in each compound. This relationship was verified for normal alcohols and for normal esters. The distribution coefficient of a compound between CO_2 and water determines how readily carbon dioxide can strip such a compound from its aqueous solution. In an homologous series of compounds, the higher molecular weight compounds have larger distribution coefficients. For equal molecular weights, esters are much more easily extracted (larger coefficients) than are alcohols.

Preliminary experiments indicate that virtually all of the constituents of orange essence which come out after ethyl butyrate on the GLC are easily and completely extracted with CO_2. More work with larger samples must be carried out to provide enough extract to study analytically the important aroma and flavor constituents.

The only phase obtained by extracting commercial orange essence with CO_2 is virtually 100% aroma constituents. Comparisons of GLC peaks of limonene and of major peaks of longer retention times indicate that the extract is 1,000-10,000 times more concentrated than the feed essence. The oily phase contains essentially no water. The aqueous extract phase is high in organics, but it is still mostly water that was dissolved in the CO_2. This phase should probably be extracted again.

At least 90% of the oily phase extract is obtained within three cycles or recirculations of the feed to contact the carbon dioxide. After 5-6 cycles there are virtually no organic compounds beyond ethyl butyrate remaining in the raffinate. The compounds whose peaks appear earlier on the GLC are much diminished in the raffinate after 5-6 cycles, and only small fractions of the initial concentrations remain.

9/11/69

ASCORBIC ACID RETENTION IN ORANGE JUICES AND CONCENTRATES AS RELATED TO TYPE CONTAINER AND STORAGE TEMPERATURE.

Owen W. Bissett and Robert E. Berry
Southern Utilization Research and Development Division
U. S. Fruit and Vegetable Products Laboratory
Winter Haven, Florida

The citrus industry has through the years advertised the nutritional values of their juices and concentrates with particular emphasis on ascorbic acid content. There have been changes in recent years both in products offered and containers used. There is need for information concerning ascorbic acid retention as influenced by these factors. This report is concerned with two products, i.e., chilled juice (SSOJ) and frozen concentrated orange juice (FCOJ). Table I indicates the containers, products and packaging conditions included in the study.

TABLE I

Container type	Sample and Treatment			
	SSOJ		Expt. FCOJ	Comm'l FCOJ
	Asceptic	Hot pack	Hot pack	Cold fill
Bottles				
Glass	x	x		
Plastic		x		
Waxed carton		x		
Polyethylene pouch				x
Cans				
Steel			x	
Aluminum			x	
Fiber, Alum 2 sides				x
Fiber, Alum inside				x
Fiber, Poly inside				x
Board, Poly. 2 sides				x

The SSOJ products were stored at 30°F., 40°F., 60°F., and 85°F. and analyzed by the indophenol method for ascorbic acid retention at 4-week intervals. The FCOJ samples were stored at -5° and 40°F. and examined in a like manner at monthly intervals.

Table II indicates that glass containers were far superior to other containerr used in ascorbic acid retention of the SSOJ samples. Ascorbic acid retention was not significantly different in asceptic and hot pack SSOJ in glass containers. Samples in plastic and carton containers at the higher storage temperatures were lost due to fermentation.

TABLE II. % ASCORBIC ACID RETENTION IN SSOJ

Weeks	Container	Storage temperature 30°F.	40°F.	60°F.	85°F.
	Glass				
4		95	97	95	93
20		94	94	90	81
32		91	94	88	--
	Plastic				
4		85	83	69	--
20		45	34	--	--
	Carton				
4		75	75	--	--
20		2	--	--	--

Table III presents representative data on ascorbic acid retention in experimental concentrates stored at -5°F. and 40°F. Reference to Table I would indicate that with the exception of the poly pouch these products should be sterile. As would be expected, samples in the polyethylene pouches stored at 40°F. were lost due to fermentation due to failure of the can closer to hermetically seal the containers. This was also true of samples in the fiber can stored at 40°F. In all cases, samples stored at -5°F. retained at least 93% of the original ascorbic acid content for 12 months and sterile products stored at 40°F. retained at least 90%.

TABLE III. % ASCORBIC ACID RETENTION IN EXPERIMENTAL FCOJ

	Container type						
	Steel		Alum.		Al.-Fiber-Al.		Poly. pouch
Months	-5°F.	40°F.	-5°F.	40°F.	-5°F.	40°F.	-5°F.
4	100	100	98	96	100	85	100
8	100	100	93	83	100	--	98
12	96	94	93	90	97	--	95

Representative ascorbic acid retention data in commercial concentrates during 9-months storage at -5°F. are presented in Table IV. All samples retained at least 91% of their original ascorbic acid content. The data indicate that aluminum is to be preferred to polyethylene and that a double polyethylene barrier is better than a single one. These studies, not yet complete, will be continued to cover a 12-month period of observation.

TABLE IV. % ASCORBIC ACID RETENTION IN COMMERCIAL FCOJ

	Container type			
Months	Al.-Fiber-Al.	Al. Fiber	Poly-Fiber	Poly-Fiber-Poly.
	-5°F.	-5°F.	-5°F.	-5°F.
3	98	100	95	98
6	98	99	94	96
9	95	97	91	93

Results thus far have indicated most container types are satisfactory for ascorbic acid retention at 40°F. and below provided containers remain intact. Glass, steel, and aluminum containers were most effective at higher temperatures.

9/4/69

RECOVERY OF PIGMENTS FROM CITRUS PEEL AND THEIR USE FOR COLOR ENHANCEMENT OF PRODUCTS.

Robert E. Berry and Theo. J. Kew
Southern Utilization Research and Development Division
U. S. Fruit and Vegetable Products Laboratory
Winter Haven, Florida

A method for obtaining coloring material from parts of the fruit other than juice would provide a source of additional pigment which would be helpful in maintaining optimum desired color in products throughout the season. A study was initiated to determine whether the coloring materials from citrus peel could be extracted and treated to provide a concentrated color extract which could be used in citrus products without being detrimental to flavor or stability of the product. In order to be more practical, a method was sought which would be applicable directly to wet peel. An extraction was developed which incorporated the following steps:

1. Grind Peel
2. Add two parts hexane, stir, filter.
3. Evaporate hexane extract to 1/3 volume.
4. Wash concentrated hexane extract with alcoholic KOH.
5. Decant solvent phase.
6. Wash with water to pH 7.5 and evaporate solvent.
7. Collect pigment concentrate and steam distill to remove d-limonene and other flavor components.

Pigment extracts were obtained using this procedure from the peel of Valencia, Pineapple, Blood, Temple, Parson Brown, and Hamlin oranges as well as Dancy tangerines. The pigment extracts were evaluated for color, quality and strength by three different methods: Bausch & Lomb Spectronic 20 colorimeter, Hunter Lab Model D45 citrus colorimeter, and visual comparison of colored juice with U.S.D.A. Standard Color Tubes. Yield of crude pigment obtained as outlined above, from the different varieties of citrus fruit is given below.

Variety	Wet peel extracted kg.	crude pigment obtained g.	yield pigment g. / peel kg.
Valencia peel	47.7	34.04	.72
flavedo*	42.5*	89.28*	2.10*
Pineapple	38.3	43.84	1.14
Blood	11.4	5.23	.47
Temple	47.7	23.83	.50
Parson Brown	27.0	21.42	.79
Hamlin	47.7	15.87	.34
Dancy (Tangerine)	18.4	23.30	1.29

* These figures are based on flavedo extracted; flavedo was about 30% of whole peel.

The color extracts were analyzed for tristimulus yellowness and redness factors using the Hunter Lab citrus colorimeter. Redness factors were highest for Dancy tangerines followed by Pineapple orange and Valencia and lowest for Hamlin. Yellowness factors were highest for Dancy tangerine followed by Valencia and Pineapple and lowest for Hamlin.

The pigment extract was easily distributed into juice or concentrate of about room temperature, and the pigment obtained from the peel from one box of oranges was sufficient to increase the U.S.D.A. color score of juice from 3 to 4 boxes of fruit. When added in these proportions, juice of U.S.D.A. standard color score 35 was raised to 36. Juice samples which had been improved in color by this method were evaluated by taste tests in homes by untrained tasters. Over 95% of a total of about 350 tasters indicated juice with added color extract was entirely acceptable.

Storage Stability

Color stability was good in samples of canned SSOJ and FCOJ to which the peel color concentrate had been added. SSOJ in both plain and enameled tin cans, with color concentrate added, retained a higher CY value than controls over a period of 12 weeks at 85°F., although there was some slight lowering of CY values after about 10 weeks. In the same samples,

CR values were considerably higher at the beginning but decreased slowly so that after 10 weeks at 85°F. CR value of experimental and control samples was about the same. Although there was no great difference in flavor stability of experimental and control SSOJ samples, the color-added samples appeared to be slightly more stable. They required 10 weeks for development of detectable differences at 85°F. while controls were different in about 6 to 8 weeks.

With FCOJ samples, at both -5°F. and 35°F., even though CR values decreased slightly with time, both CR and CY values remained considerably higher in experimental than in control samples for more than 12 weeks. Both experimental and control samples developed detectable flavor differences in 8 weeks at 35°F.

UNITED STATES DEPARTMENT OF AGRICULTURE

AGRICULTURAL RESEARCH SERVICE

LIST OF CITRUS PUBLICATIONS

AND PATENTS

(September 1, 1968 - August 31, 1969

Reprints of publications may be obtained without cost by addressing request to the Laboratory listed.

Patents may be obtained only by purchase from the U. S. Patent Office, Washington, D. C. 20250, for 50 cents each.

9/29/69

SOUTHERN UTILIZATION RESEARCH AND DEVELOPMENT DIVISION

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SOUTHERN UTILIZATION RESEARCH AND DEVELOPMENT DIVISION

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SOUTHERN UTILIZATION RESEARCH AND DEVELOPMENT DIVISION

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WESTERN UTILIZATION RESEARCH AND DEVELOPMENT DIVISION

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Albany, California 94710

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WESTERN UTILIZATION RESEARCH AND DEVELOPMENT DIVISION

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263 South Chester Avenue
Pasadena, California 91106

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UNITED STATES DEPARTMENT OF AGRICULTURE
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REPLY TO:
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NEW ORLEANS, LA. 70119

September 15, 1969

We are pleased to invite you to attend the 1969 Conference on Citrus Chemistry and Utilization scheduled to take place in Winter Haven, Florida, at the Landmark Motor Lodge, on October 17, 1969.

This Conference is sponsored annually by the Southern Utilization Research and Development Division of the Agricultural Research Service, U. S. Department of Agriculture. Its principal purpose is to acquaint representatives of the citrus industry with research developments in the broad area of processing, marketing, and utilization, and to provide for exchange of information that will benefit the industry and future research.

Registration will begin at 8:00 a.m., and the Conference proper will begin at 9:00 a.m. The meeting is open to anyone interested. Everyone in attendance is invited to actively participate in the discussions. No registration fee is required.

A program is enclosed together with a card for registration and reservation purposes.

C. H. Fisher

C. H. Fisher
Director

2 Enclosures

1969 CONFERENCE ON CITRUS CHEMISTRY AND UTILIZATION

October 17, 1969
LANDMARK MOTOR LODGE
Winter Haven, Florida

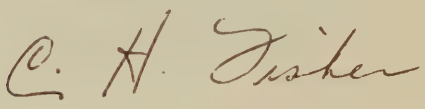
Southern Utilization Research and Development Division
Agricultural Research Service
United States Department of Agriculture
New Orleans, Louisiana

FOREWORD

Chemical and utilization research strive to develop new ideas, products, and processes to help the growers, industry, and the consumer.

The Conferences on Citrus Chemistry and Utilization are sponsored by the Southern Utilization Research and Development Division of USDA's Agricultural Research Service to report research developments in the broad area of processing, marketing, and utilization, and to provide for exchange of information that will benefit the industry and future research.

Those interested are cordially invited to attend and participate in the discussions. Dr. M. K. Veldhuis, Chief, U. S. Fruit and Vegetable Products Laboratory, 600 Avenue S N. W., Winter Haven, Florida, will be pleased to make your hotel reservation. No registration fee will be required.



C. H. Fisher, Director

Southern Utilization Research and Development Division
Agricultural Research Service
U. S. Department of Agriculture

GENERAL CHAIRMAN:

Frederic R. Senti, Deputy Administrator, Nutrition, Consumer and Industrial Use Research, Agricultural Research Service, U. S. Department of Agriculture, Washington, D. C.

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Shirley T. Saucier

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Ellen S. Pearce

PROGRAM

Landmark Motor Lodge
Winter Haven, Florida

Friday, October 17, 1969

8:00 A. M. Registration

9:00 A. M. WELCOME

C. H. Fisher, Director
Southern Utilization Research
and Development Division
New Orleans, Louisiana

OPENING REMARKS BY GENERAL CHAIRMAN

Frederic R. Senti, Deputy Administrator
Nutrition, Consumer and Industrial Use
Research
Agricultural Research Service
U. S. Department of Agriculture
Washington, D. C.

Presiding: **George W. Truitt,** Foods Division, Coca Cola Co., Orlando, Florida

THE EFFECTS OF STORAGE CONDITIONS ON THE LIPID COMPOSITION OF COMMERCIALLY PREPARED ORANGE JUICE

Steven Nagy, Harold E. Nordby, and Howard L. Dinsmore
U. S. Fruit and Vegetable Products Laboratory
Winter Haven, Florida

Canned single strength orange juice and the same product chilled in glass containers were obtained from local citrus processing plants and subjected to varying storage conditions over an 18-month period. Glass chilled samples were stored at 40°F. and 85°F. while canned samples were stored at 0°F. and 85°F. Samples from each batch were taken periodically over this period, freeze dried and comparatively analyzed for their neutral lipid and phospholipid content.

The total fatty acid profiles of these different temperature stored samples did not show any marked percentage difference except for linolenic acid which showed a slight 2%

decreased. Both canned and glass stored juice maintained at 85°F. showed a substantial increase in free fatty acids as evidenced by TLC densitometry. The quantitative change in the distribution patterns of neutral lipids and phospholipids between these two temperature stored samples and the significance of this change will be discussed.

COMPOSITION AND INHERITANCE OF FLAVANONES IN CITRUS FRUIT

Roger F. Albach and George H. Redman
U. S. Food Crops Utilization Research Laboratory
Weslaco, Texas

A thin-layer chromatographic survey was made of flavanones present in 41 citrus varieties representing 18 recognized species of citrus. In addition, 49 hybrids of 18 different crosses were also surveyed. An analysis of the data has shown that a qualitative and quantitative consistency of flavanone composition, with minor variations, is characteristic of individual citrus species and crosses. Rules governing the inheritance of citrus flavanones were deduced from the composition of known hybrids. These rules, coupled with compositional data, were used to evaluate the probably taxonomic relationships of various citrus varieties and species.

The rules governing the inheritance of the bitter flavanones may be of value to the citrus breeder. A knowledge of the flavanone composition characteristic of a particular citrus species or cross may be useful to the processing industry for determining the varietal composition of citrus products.

APPLICATION OF ENZYME RESEARCH IN CITRUS PROCESSING

Joseph H. Bruemmer, Robert A. Baker, and Bongwoo Roe
U. S. Fruit and Vegetable Products Laboratory
Winter Haven, Florida

An approach to a process for decreasing acidity of citrus fruit was developed from analysis of malic and isocitric dehydrogenase reactions that lead respectively to the formation and degradation of citric acid. Malic dehydrogenase is inhibited by a slight increase in the redox state of its coenzyme. Anaerobic treatment of freshly picked fruit rapidly increases the redox state and decreases acidity of the juice extracted from the fruit.

A second application of the enzyme perspective to citrus processing is the approach to a process of stabilizing juice cloud without heating. Juice-soluble pectin is not required for stability but contributes to instability through degrada-

tion by pectinesterase. Removing the substrate for this reaction from the juice prevents degradation and stabilizes the cloud.

RECENT DEVELOPMENTS IN SRS CONSUMER RESEARCH ON CITRUS PRODUCTS

Margaret E. Weidenhamer, Chief
Special Surveys Branch
Standards and Research Division
Statistical Reporting Service
U. S. Department of Agriculture
Washington, D. C.

Data of interest to citrus utilization researchers from sensory evaluation tests in a laboratory setting and surveys of homemakers' opinions will be discussed. "Taste-testing" experiments have been made with orange juices varying in factors such as sweetness, amount of peel oil, and degrees Brix. Most of this work has been conducted among an untrained panel of USDA employees in Washington, D. C. However, an extension of experiments on preferences among 12 orange juices varying in peel oil content and sweetness level was conducted under contract in Chicago. Tests were run in a mobile laboratory to ascertain reactions among both adults and children, and a second phase was conducted among a panel of households using three of the juices to determine whether use under normal in-home conditions would alter the relative preference ratings. Other studies included questions on general reactions to selected citrus products and new product concepts.

BITTERNESS, SWEETNESS, AND THE STRUCTURE OF CITRUS GLYCOSIDES

R. M. Horowitz and B. Gentili
Fruit and Vegetable Chemistry Laboratory
Western Utilization Research and Development Division
Pasadena, California

Certain citrus fruits contain intensely bitter flavanone glycosides, all of which have as their sugar component the disaccharide neohesperidose. Other citrus fruits contain tasteless glycosides in which the sugar component is the isomeric disaccharide rutinose. Isomerism in the sugar moiety is thus of crucial importance in determining bitterness or tastelessness in this group of compounds. When alterations are made at selected sites in the bitter flavanone glycosides, the product may be bitter, bitter-sweet, sweet, or tasteless. Of particular interest are the dihydrochalcone derivatives, several of which are intensely sweet. A review

of recent findings in this field will be given together with a brief summary of taxonomic implications stemming from the distribution of these glycosides in citrus.

12:00 Noon Luncheon

1:30 P. M.

Presiding: **C. Byron Smith,** Plymouth Citrus Products Coop., Plymouth, Florida

ANALYSIS OF STORED INSTANT ORANGE JUICE AND TASTE EVALUATION OF SOME STORAGE DECOMPOSITION PRODUCTS

Philip E. Shaw, James H. Tatum, Theo. J. Kew, and Robert E. Berry
U. S. Fruit and Vegetable Products Laboratory
Winter Haven, Florida

Eighteen decomposition products were identified from instant orange juice after prolonged storage at elevated temperatures. Their taste thresholds were determined and ranged from 3 p.p.m. to greater than 200 p.p.m. The 18 products included 8 furans, 3 pyrroles, 4 acids, one α-pyrone derivative, β-butyrolactone, and methylcyclopentenolone. The pyrone derivative and one acid have not previously been reported in IOJ. To prove identification, syntheses were developed for several compounds. Quantities in IOJ when off-flavor first develops have been estimated. Additive effects cause some compounds to influence flavor in spite of their presence at concentrations below individual taste thresholds. Some browning inhibitors increased IOJ shelf life.

LIMONIN IN FLORIDA CITRUS: A PRELIMINARY REPORT

W. Clifford Scott
U. S. Fruit and Vegetable Products Laboratory
Winter Haven, Florida

Analyses for limonin were made by the TLC method of Dreyer and Maier of the Pasadena laboratory. All varieties of citrus examined were found to contain at least a trace of limonin at early maturity. Only the Marsh grapefruit, K-Early and Murcott orange juices contained as much as 3 p.p.m. (reported threshold of limonin flavor) during their normal period of harvest. No difference in limonin content of orange juices was found between low and high extraction pressures. Limited tests indicated that freeze damage to fruit on the tree increased limonin content of juice.

LIQUID CO_2 EXTRACTION OF ORANGE ESSENCE

John M. Randall
Engineering and Development Laboratory
Western Utilization Research and Development Division
Albany, California

The flavor components of orange juice or commercial orange essence are easily and completely extracted by direct contact with liquid carbon dioxide. Typical extraction takes place at room temperature and 800-900 p.s.i.g. pressure. The extract essence is recovered by evaporating the CO_2 at 3°C.-5°C. above the extraction temperature. Higher molecular weight compounds with low water solubility almost completely stripped from the feed in two or three liquid CO_2 contact cycles. Even most of the low boiling compounds with low to high solubility in water can be 90%-100% extracted in three to six contact cycles. Ethanol and water are extracted quite slowly.

ASCORBIC ACID RETENTION IN ORANGE JUICES AND CONCENTRATES AS RELATED TO TYPE CONTAINER AND STORAGE TEMPERATURE

Owen W. Bissett and Robert E. Berry
U. S. Fruit and Vegetable Products Laboratory
Winter Haven, Florida

Samples of single strength orange juice and 45° Brix concentrates were stored in various types of containers at 85°F., 40°F., 32°F., or -5°F. SSOJ in glass retained over 80% ascorbic acid after 20 weeks at 85°F., with much higher retention at 32°F. In plastic and cardboard cartons, ascorbic acid dropped sharply. With FCOJ at -5°F., ascorbic acid retention was 90% or higher in steel, aluminum, and aluminum-fiber composite cans. Composite containers failed with FCOJ at 40° F., but the other were satisfactory. FCOJ in polyethylene pouches retained 95% ascorbic acid after one year at -5°F. These samples, packaged without benefit of heat sterilization, fermented at 40°F., however. Results indicate most container types are satisfactory for ascorbic acid retention at 40°F. and below, provided container remains intact. Glass, steel, and aluminum containers were effective at higher temperatures.

RECOVERY OF PIGMENTS FROM CITRUS PEEL AND THEIR USE FOR COLOR ENHANCEMENT OF PRODUCTS

Robert E. Berry and Theo. J. Kew
U. S. Fruit and Vegetable Products Laboratory
Winter Haven, Florida

Pigments were concentrated from peel of several varieties of oranges and tangerines. Wet pel or flavedo was extracted with hexane, the extract concentrated, washed with methanolic KOH, further concentrated, and the solvent removed by steam distillation. Yields of pigment ranged from .34 to 1.29 g. per kg. of peel. Analyses for yellowness (Y) and redness (R) factors indicated both were highest from Dancy tangerine and lowest from Hamlin oranges. Among commercial orange varieties, Pineapple had highest R factors and Valencia highest Y factors. Most pigment concentrates were effective at dilution of 1/3500 in improving product color without affecting flavor.

ADJOURNMENT

There will be a meeting of the Florida Section, Institute of Food Technology in Winter Haven, Florida, on Thursday evening, October 16, 1969. Dr. C. H. Browning, Dean for Resident Instruction, Institute of Food and Agricultural Sciences, University of Florida, will be the speaker.

Your attention is called to the following meeting:

20th Annual Citrus Processors' Meeting
University of Florida Citrus Experiment Station
Lake Alfred, Florida (Zip Code 33850)
Thursday, October 16, 1969

Registration will begin at 9:30 A. M.

The Station is only a few miles from the U. S. Fruit and Vegetable Products Laboratory. Further details of this meeting may be obtained at the above address.